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## 8 Effects of Metal Pollutants on Decomposition Processes in Terrestrial Ecosystems with Special Reference to Fungivorous Soil Arthropods

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### ABSTRACT

Environmental quality standards for soils are often derived from laboratory-based experiments that disregard interactions between species. These interactions make effects of pollutants in the field difficult to predict from single species tests. A good example is the complexity of invertebrate–microbial relationships. One of the best-studied interactions is that between fungal hyphae and fungivorous springtails (Insecta: Collembola). Collembola are “primitive” wingless microarthropods that are important components of most terrestrial decomposer communities. This review concentrates on the relationships between fungal hyphae and Collembola. It aims to show how knowledge of the interactions between species helps us to understand the effects of metal pollutants on individuals, populations, and ecosystem processes such as decomposition of plant remains. The review concludes with a discussion of the ecological implications for invertebrate–microbial relationships of setting “95% protection levels” for metals in soils.

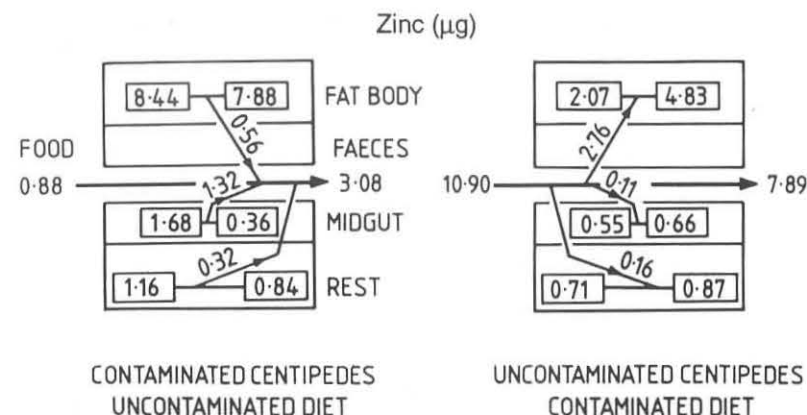
### 8.1 INTRODUCTION

The routes of exposure of soil animals to metal pollutants in contaminated terrestrial ecosystems are extremely complex. On an ecosystem level, pollution of soils can arise from aerial deposition from sources such as smelting works or car exhausts, or from persistence of contamination following closure of metalliferous mines. However, the factor which in many cases determines the severity of the disruption of normal ecological processes in soil and leaf litter ecosystems, is the extent to which metals are accumulated and transferred between soil organisms (Hopkin, 1989). It is important to understand these

processes at every level of organisation from the ecosystem down to individual organelles (Figures 8.1 and 8.2). Only then will it be possible to set critical concentrations for metals that we can be confident will protect the majority of species (Van Straalen and Ernst, 1991; Van Straalen, 1993).

Understanding all aspects of metal dynamics in soil and leaf litter may seem like an impossible task. Nevertheless, much progress has been made in recent years and unifying concepts are beginning to emerge. For example, numerous studies have shown that copper and lead have an extremely high affinity for organic matter and are much less mobile than cadmium and zinc at normal to slightly acid pHs (Scokart *et al.*, 1983; Bergkvist *et al.*, 1989; see Chapter 9, this volume).

Several ecotoxicologists have described processes of metal detoxification that appear to be common across a wide range of species (Depledge and Rainbow, 1990; Rainbow *et al.*, 1990; Beeby, 1991; Dallinger, 1993). Among these is the propensity of particular groups of terrestrial invertebrates to retain metals in organs associated with the digestive system (Hopkin, 1989), and to store these contaminants as intracellular granules of three main types (Hopkin *et al.*, 1989; Hopkin, 1990a). However, it is more difficult to predict the effects of metal contamination on populations due to the complexity of soil

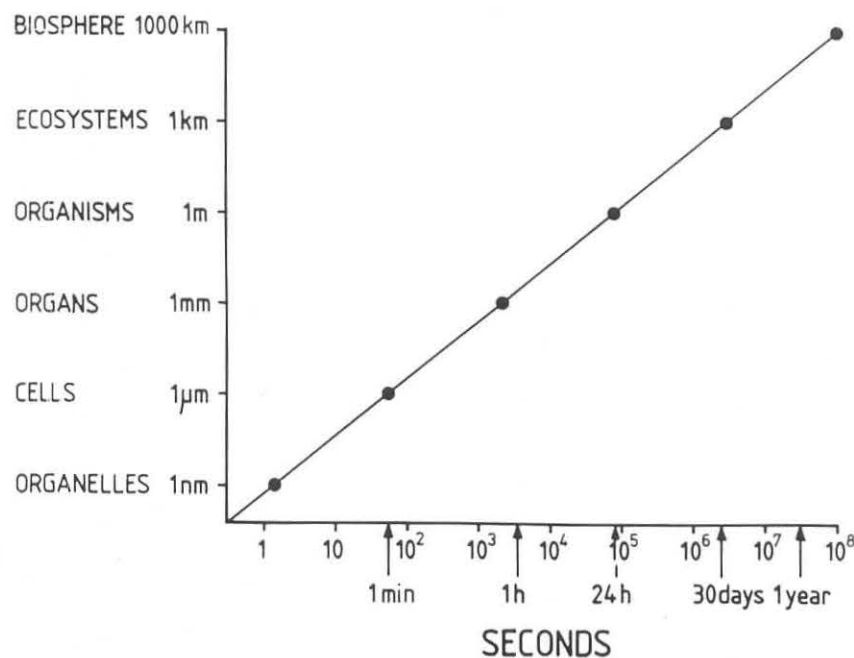


**Figure 8.2.** Net fluxes in amounts of zinc ( $\mu\text{g}$ ) through *Lithobius variegatus*, and between the midgut, fat body (sub-cuticular tissues) and "rest" of the tissues of centipedes from a contaminated and an uncontaminated site. Centipedes were fed over a 28 day period on the hepatopancreas of six uncontaminated or six contaminated specimens of the woodlouse *Oniscus asellus* collected from the same sites (mean of seven centipedes in both cases). The value given in the left-hand box for each tissue fraction represents the amount of zinc estimated to have been present at the start of the experiment. The values in the right-hand boxes represent the amounts present at the end. Most of the zinc excreted in the faeces of contaminated centipedes was lost from the midgut ( $1.32 \mu\text{g}$ ). Most of the net zinc assimilation by uncontaminated centipedes was accounted for by an increase in the amount in the fat body ( $2.76 \mu\text{g}$ ). After Hopkin and Martin (1984)

and leaf litter communities. It is important to quantify these effects since absence of particular species may disrupt decomposition or other important ecological processes.

In this chapter these topics will be discussed using, as the main example, the importance of fungal hyphae as a critical pathway of exposure to metals for fungivorous soil and leaf litter arthropods. After a general discussion on invertebrate-microbial interactions during decomposition (Section 8.2), the effects of metals on microbial processes (Section 8.3) and Collembola will be described (Section 8.4). Collembola are wingless "primitive" insects that are extremely common in soil and leaf litter (Figures 8.3, 8.4). Transfer of metals to the predators of Collembola will be covered in Section 8.5, and the chapter concludes (Section 8.6) with a discussion of the ecological implications of setting critical concentrations for individual metal pollutants in soils.

The literature on metals and microorganisms is huge. Several excellent and comprehensive reviews on the subject have been published in recent years (e.g. Duxbury, 1985; Gadd, 1988, 1990; Hughes and Poole, 1989; Wilkins, 1991) but interactions between microorganisms, invertebrates and metal pollution have been less well covered. Consequently, this chapter will concentrate on



**Figure 8.1.** Relationship between complexity and size of natural systems and "compartments" (= "black boxes") and typical response times to metal "insults"

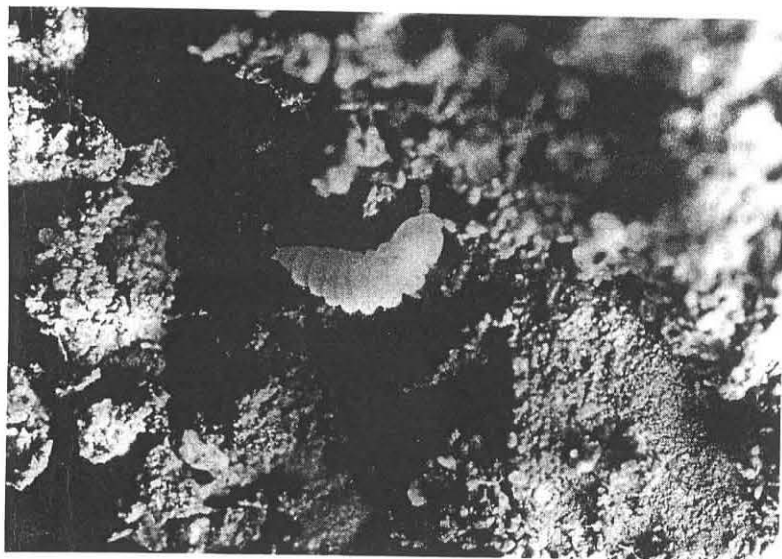


Figure 8.3. A euedaphic (soil-dwelling) collembolan (*Onychiurus armatus*) of 2 mm in length

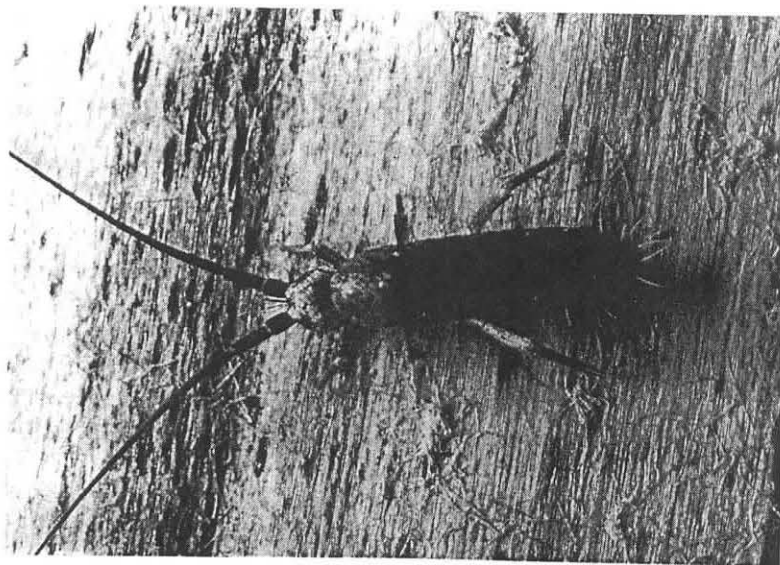


Figure 8.4. An edaphic (litter-dwelling) collembolan (*Tomocerus longicornis*) of 5 mm in length

these interactions, especially food chain transfer and effects of metals in relation to the feeding biology of fungivorous soil arthropods.

## 8.2 INVERTEBRATE-MICROBIAL INTERACTIONS DURING DECOMPOSITION

Many soil and leaf litter invertebrates including isopods, millipedes, termites and mites, as well as Collembola, have evolved in intimate association with fungi and bacteria. One role of microorganisms in the association is to make nutrients in dead plant material available for assimilation by supplying enzymes such as cellulases which the animals are unable to produce themselves (Gunnarsson and Tunlid, 1986; Gunnarsson, 1987; Hopkin and Read, 1992; Hopkin, 1993a). A well-known example is that of leaf-cutting ants which culture fungus in "gardens" which they feed to their larvae (Holldobler and Wilson, 1990). Most early colonisers of the land in the Devonian and Carboniferous periods probably grazed fungal hyphae from the surfaces of leaf litter. The hyphae acted as an "external rumen" by degrading plant material which the digestive systems of the invertebrates were unable to break down (Price, 1988; Pearce, 1989; Little, 1990).

Fungus is a rich source of readily-available nutrients (Cromack *et al.*, 1977) and there are numerous species of Collembola which feed extensively on hyphae in soil and leaf litter (Van Straalen, 1989; Faber, 1991a,b; Hedlund *et al.*, 1991). *Sinella curviseta* grazes hyphae from roots and has been put forward as a potential biological control agent of the fungus that causes wilt disease of cucumber seedlings (Nakamura *et al.*, 1992). However, grazing of the vesicular-arbuscular mycorrhiza (VAM) *Glomus fasciculatus* by *Folsomia candida* leads to a reduction in growth rate of leek (*Allium porrum*) (Warnock *et al.*, 1982). The volume edited by Fitter (1985) includes several other examples of interactions between mycorrhizae and Collembola.

There is no evidence that Collembola possess a permanent symbiotic microflora like that of termites (Ponge, 1991a). The bacteria present in the lumen of the digestive system of Collembola are derived from their food although these microbes may proliferate in the gut and contribute enzymes to digestive processes before being lost in the faeces. Bacteria associated with food material may also be digested during gut passage.

Thus, if fungal hyphae were to accumulate metals to higher concentrations than the material on which they were growing, the potential exists for much greater transfer of pollutants to fungivores than might be predicted from analysis of pooled samples of leaf litter.

Soil invertebrates are responsible directly for only about 5–10% of the chemical decomposition of leaf litter (Petersen and Luxton, 1982). However, they act as "catalysts" by stimulating the activities of bacteria and fungi which conduct the majority of chemical decomposition (Anderson and Ineson, 1984;

Anderson 1988; Shaw *et al.*, 1991). They do this by fragmenting leaf litter into small particles which are voided as faecal pellets. These pellets provide a more favourable substrate for microbial breakdown (Eisenbeis and Wichard, 1987). Decomposition is promoted further if the faeces are deposited in deeper, moister litter layers where decomposition is faster and bacterial and fungal spores can germinate (Hassall *et al.*, 1986a; Hames and Hopkin, 1989; Van Wensem, 1989).

The presence of invertebrates has been shown to have a profound effect on mineralisation and subsequent fluxes of major nutrients (Griffiths *et al.*, 1989; Morgan *et al.*, 1989; Verhoef and Brussard, 1990; Faber and Verhoef, 1991). Clearly, they must also influence fluxes of metal pollutants, although as far as the author is aware, this has not been quantified in detail. Van Straalen *et al.* (1985) demonstrated that food chain transfer of lead by the collembolan *Orchesella cincta* was very small (about 11 times the population standing pool per year) in comparison to the flux of lead through consumption and defecation (about 10 000 times the standing pool per year).

One of the characteristic features of metal-contaminated ecosystems is a reduction in the rate of decomposition of dead plant material. Accumulation of leaf litter occurs in deciduous woodlands (Coughtrey *et al.*, 1979) and coniferous forests (Bengtsson *et al.*, 1988a) and is due primarily to a reduction in the activities of microorganisms and litter-consuming invertebrates (Strojan, 1978), not to an increase in the amount of litter falling onto the forest floor (Jackson and Watson, 1977). Bengtsson *et al.* (1988a) showed that the rate of leaf litter decomposition in metal-contaminated sites near to a brass mill in Sweden was only about one-tenth of the rate in uncontaminated areas. This difference was attributed to decreases in the activities of microorganisms, Collembola, enchytraeids and mites as a result of metal pollution.

### 8.3 METALS AND MICROORGANISMS

The effects of metal pollution on microbial communities are extremely difficult to quantify (Hughes and Poole, 1989; Tyler *et al.*, 1989). Many studies have examined species assemblages (treating the microbial community as a "black box"), studying macro effects such as reduction or stimulation of respiration, or rates of decomposition of simple substances such as sugars (Ruhling and Tyler, 1973; Tyler, 1974, 1975; Bond *et al.*, 1976; Strojan, 1978; Hattori, 1989, 1991; Wilke, 1991). Babich *et al.* (1983) proposed that the inhibition of microbe-mediated ecological processes (mineralisation of carbon, respiration and nitrification) could be used to quantify the sensitivity of natural ecosystems to pollutants. Such studies indicate the effects of metal pollutants on *processes*. However, the black box approach disguises changes in the composition of species. For example, Nordgren *et al.* (1983) showed that the numbers of colony-forming units of microfungi did not change along a

gradient of metal pollution but that the composition of species changed drastically.

Some researchers have isolated species for study. Clint *et al.* (1991) measured a wide range of species-specific differences in influx rates of  $^{137}\text{Cs}$  into fungal hyphae. However, results derived from this approach must be treated with care if they are extrapolated to the complex situation that occurs in the field. For example, Doelman and Haanstra (1979a,b) showed that there was an order of magnitude difference between sandy and peaty soils in the concentration of lead that significantly reduced respiration and dehydrogenase activities of microbes.

Total microbial biomass in soil may be reduced by metal pollution (Bisessar, 1982). For example, soil biomass carbon as a proportion of total carbon was reduced from its normal level of around 2%, to 1% in smelter-polluted soil in Sweden (Baath *et al.*, 1991). Species that are tolerant to metal pollution may proliferate under such circumstances (Duxbury and Bicknell, 1983). Indeed, there are several species of fungi that are tolerant to copper (Baath, 1991; Table 8.1). Metal pollution may lead to selection for resistant (i.e. genetically distinct) strains (Jordan and Lechevalier, 1975; Doelman and Haanstra, 1979c). Selection may be rapid and can occur by transfer of metal resistance genes between species (Top *et al.*, 1990).

The basis of resistance to cadmium, copper or zinc may be duplication of the gene that codes for the detoxifying protein metallothionein (Gadd and

**Table 8.1.** Concentration of copper ( $\text{mg Cu l}^{-1}$ ) in media giving 50% reduction of radial growth rate ( $\text{LD}_{50}$ ), and highest Cu concentration where growth was present after 1 month incubation. Highest concentration tested was  $400 \text{ mg Cu l}^{-1}$ . Swedish isolates from soil were used unless otherwise stated (reproduced by permission of the British Mycological Society from Baath 1991)

Fungal species	$\text{LD}_{50}$	Growth
<i>Cordyceps militaris</i> (L.:Fr.) Link CBS 110.70	>400	400
<i>Paecilomyces farinosus</i> (Holm:Fr.) A. H. Brown & G. Smith	>400	400
<i>Verticillium lecanii</i> (Zimm.) Viégas CBS 546.81	263	200
<i>Beauveria bassiana</i> (Balsamo) Vuill.	204	400
<i>Metarhizium anisopliae</i> (Metschn.) Sorok. CBS 459.75	195	200
<i>Verticillium chlamydosporium</i> Goddard	174	200
<i>Aureobasidium pullulans</i> (de Bary) Arnaud	160	200
<i>Verticillium suchlasporium</i> Gams & Dackman	105	200
<i>Microdochium bolleyi</i> (Sprague) de Hoog & Hermanides-Nijhof	74	100
<i>Fusarium oxysporum</i> Schlecht. CBS 267.50	72	100
<i>Alternaria alternata</i> (Fr.:Fr.) Keissler	71	200
<i>Mucor hiemalis</i> Wehmer CBS 201.65	<72	100
<i>Rhizopus oryzae</i> Went & Prinsen Geerligs	<59	100
<i>Paecilomyces lilacinus</i> (Thom) Samson	54	100
<i>Paecilomyces variotii</i> Bain.	<50	25
<i>Trichoderma polysporum</i> (Link:Fr.) Rifai	<41	100



White, 1989). The consequence of the duplication is that metallothioneins may be produced in larger amounts in response to a metal "insult". There may also be increased secretion of metal-binding ligands which precipitate metals extracellularly (Wood *et al.*, 1984; Mullen *et al.*, 1992), or a decrease in the rate of assimilation across the cell wall (Budd, 1991).

Fungal hyphae have a remarkable ability to accumulate metals to exceptionally high concentrations (Gadd, 1988, 1990). For copper, much of the accumulated metal is adsorbed onto the external surfaces of the hyphae (Gadd and White, 1989). Concentrations of more than  $3 \text{ mg Cu g}^{-1}$  have been measured in *Verticillium bulbillosum* grown on a medium containing only  $150 \text{ } \mu\text{g Cu ml}^{-1}$  (Bengtsson *et al.*, 1983). Mitani and Misić (1991) measured levels of  $16 \text{ mg Cu g}^{-1}$  of mycelium ( $=1.6\%$  of the dry weight) in *Penicillium* sp. grown on a medium containing  $1 \text{ mg Cu ml}^{-1}$ ; at least  $65\%$  of this copper was present on the surface. Hopkin (1993a) measured  $6720 \text{ } \mu\text{g Cu g}^{-1}$  dry weight in fungal hyphae scraped from a leaf to which copper nitrate had been applied, to give a total leaf concentration of only  $107 \text{ } \mu\text{g g}^{-1}$ . There is strong evidence for the presence of energy-dependent transport mechanisms for other essential elements such as zinc (Starling and Ross, 1991).

Hopkin (1993a) has suggested that basidiomycete fungal hyphae may need to accumulate high concentrations of essential metals since they undergo massive growth dilution of nutrients when sporophores are produced. Non-essential elements such as cadmium, which are also accumulated to very high concentrations by hyphae (Gadd and White, 1989), may follow similar biochemical pathways to essential metals such as copper and zinc. For example, the transport system for zinc in *Penicillium notatum* is inhibited competitively by cadmium, but not by copper or other cations (Starling and Ross, 1991).

Many fungi in soil and leaf litter form mycorrhizal associations with the roots of higher plants (Newman, 1988; Brundrett, 1991). The hyphae are extremely important in enhancing nutrient uptake and, in exchange, may obtain up to  $40\%$  of the photosynthate produced by the host plants (Gehring and Whitham, 1991). In white clover (*Trifolium repens*), more than  $50\%$  of the copper assimilated by the plants was via its mycorrhizal fungus (Li *et al.*, 1991). In two species of grass, VAM infection "protected" the plants against zinc poisoning (Dueck *et al.*, 1986). However, in pigeon pea, VAM infection increased assimilation of zinc by the plants (Wellings *et al.*, 1991).

In natural situations, it is difficult to quantify the potential effects of metal pollution on these associations (Wilkins, 1991). For example, in experiments on the effects of lead on six-month old loblolly pine (*Pinus taeda*) and its ectomycorrhizum *Cenococcum geophilum*, growth was greatest at the lowest and highest concentrations of lead in the soil with least growth at intermediate concentrations (Chappelka *et al.*, 1991). Mycorrhizal fungi of pine are involved also in the decomposition of old pine needles when the fine roots

penetrate among them. Indeed, there is evidence that their presence may inhibit bacterial colonisation (Ponge, 1991b).

Tyler (1991), in a simple but elegant experiment, examined the effects on fungi of removal of the annual litter fall in a Swedish beech forest. Removal of litter for two consecutive years increased the sporophore production of mycorrhizal *Russula* species in both years whereas sporophore production by most decomposer agarics was greatly reduced. It is possible that the lower availability of nutrients stimulated the development of ectomycorrhizae. Thus in metal-polluted sites where the rate of decomposition of leaf litter is reduced, growth of metal-tolerant fungal hyphae may be stimulated, with increased potential for food chain transfer of pollutants. However, at present, it is difficult to come to any firm conclusions as to the effects of metal pollution on fungal mycorrhizae.

In contrast, Stahl and Christensen (1992) showed that for non-mycorrhizal species, competition between different soil fungi was greatest when they were colonising resource-rich habitats in comparison to nutrient-poor media.

#### 8.4 EXPOSURE OF FUNGAL-FEEDING COLLEMBOLA TO METALS

Collembola may reach densities of several thousand per square metre in temperate woodlands. Many species consume a diet that is mainly, if not exclusively, composed of fungal hyphae. Collembola graze fungus from the surfaces of plant roots, soil particles and leaf litter (Saur and Ponge, 1988; Faber, 1991a; Ponge, 1991a). The grazing can be quite selective in terms of species preferred (Hassall *et al.*, 1986b; Schultz, 1991). It can also alter the extent to which leaf litter is colonised by different species of fungi (Parkinson *et al.*, 1979; Seastedt, 1984), may inhibit or stimulate hyphal growth (Hanlon and Anderson, 1979; Hanlon, 1981; Faber, 1991b; Leonard and Anderson, 1991a,b; Faber *et al.*, 1992) and may have important implications for the success or failure of populations of particular species of Collembola in metal-contaminated sites.

The study of food preferences is important in understanding the population dynamics of Collembola. Usher *et al.* (1982) reported that the growth rate of *Folsomia candida* depended on the species of fungus on which it was fed. However, more research is needed on food preferences since it is not clear to what extent collembolan diets are dictated by availability rather than choice in soil and litter microhabitats (Gilmore and Raffensperger, 1970; Anderson and Healey, 1972; Vegter, 1983).

*Isotoma olivacea* was more abundant in a lead-polluted site in Norway than in adjacent uncontaminated areas because it was able to take advantage of the lack of competition from other species of Collembola which were more sensitive to the metal (Hagvar and Abrahamsen, 1990). Similar community

effects were also observed near to the Gusum brass mill in Sweden (Bengtsson and Rundgren, 1988).

These phenomena may be due to species-specific differences in sensitivity to the same levels of pollution, or to a choice of diets containing different concentrations of metals. For example, *Folsomia fimetaria* dominates *Isotomiella minor* in metal-polluted soils due to a combination of being able to avoid consuming metal-rich fungi, the prevalence of its preferred fungal diet in the polluted sites and, possibly, more efficient detoxification mechanisms (Bengtsson and Rundgren, 1988; Tranvik and Eijsackers, 1989). Indeed, total densities of Collembola may be higher in metal-polluted woodlands if the thick accumulation of leaf litter provides a refuge from predation (Hopkin *et al.*, 1985).

Van Straalen (1989) calculated that Collembola in a Dutch pine forest assimilated the equivalent in energy terms of 6% of the annual litterfall (although much of this must have been via consumption of fungal hyphae). The grazing activities of Collembola increase nitrogen mobilisation from pine litter, but this effect is often overlooked as most of the nitrogen is assimilated rapidly by ectomycorrhizal fungi on tree roots (Faber and Verhoef, 1991). Thus by implication, Collembola may be of great importance in releasing metals bound in metal-rich fungi and making these available to trees.

Two species of Collembola, *Onychiurus armatus* and *Orchesella cincta*, have been examined in the greatest detail with regard to their metal dynamics. The research has been conducted primarily by groups in Sweden and The Netherlands respectively. Earlier research by these workers was reviewed by Hopkin (1989) and Joosse and Verhoef (1987) but the summary below includes more recent publications which have shed new light on the effects of metal pollution on Collembola.

#### 8.4.1 ONYCHIURUS ARMATUS

*Onychiurus armatus* (Figure 8.3) is edaphic and lives permanently in the humus layer or mineral soil where it ingests mycorrhizal and/or saprophytic fungal hyphae or spores (Faber, 1991a). This species has been studied extensively in relation to the effects of copper, lead and zinc pollution from a brass mill at Gusum in Sweden in a series of papers by Bengtsson, Rundgren and co-workers at the University of Lund. *Onychiurus armatus* locates fungal hyphae in soil by following concentration gradients of volatile compounds released from the mycelium (Bengtsson *et al.*, 1991). These compounds are in the range C<sub>5</sub> to C<sub>18</sub>, and in olfactometer experiments are released at a rate of about 250 pg h<sup>-1</sup> from a patch of fungus 175 mm<sup>2</sup> representing about 400 µg of mycelium (Bengtsson *et al.*, 1988b). In these experiments, the collembolan showed a distinct preference for particular fungal species, although the order of preference changed depending on whether the fungus was cultivated on agar or soil.

*Verticillium bulbillosum* was most attractive when fungi were cultured on agar (Bengtsson *et al.*, 1988b). This species of fungus contained concentrations of copper and lead of at least an order of magnitude greater than the substrate on which they were growing. Experiments on the growth of *Onychiurus armatus* fed on this diet showed that reductions could be detected at concentrations of copper and lead in the collembolans that were found in field populations near to the brass mill. However, despite a prediction of extinction at the site (Bengtsson *et al.*, 1985a), Collembola are able to survive at the site because the situation in the field is more complex than in the laboratory. For example, *Onychiurus armatus* can tolerate higher levels of metals if plenty of protein-rich food is supplied (Bengtsson *et al.*, 1985b). A further interesting observation was that the collembolans performed best when their food was slightly contaminated with metals (known as "hormesis"; Stebbing, 1982). This effect may have been due to the elimination of a metal-sensitive parasite, or in response to a copper deficiency, or due to the stimulation of fungal growth following an increase in available nutrients after lysis of metal-sensitive microorganisms.

The Swedish work has shown that fungal hyphae accumulate metals to much higher concentrations than those in the substrate. Collembola feed on the hyphae and accumulate metals. The metals inhibit growth and reproduction at tissue concentrations that can be found in the field, but these effects may be mitigated by the presence of protein-rich food and/or avoidance by the Collembola of the most contaminated fungus. The pollution can also alter the normal species composition of an area, favouring those able to tolerate the metals.

#### 8.4.2 ORCHESELLA CINCTA

*Orchesella cincta* is edaphic and lives among recently fallen leaf litter where it feeds on saprophytic fungal hyphae and spores (Faber, 1991a). The species has been studied in the field and laboratory by Van Straalen and co-workers at the Vrije University, Amsterdam. The ease with which *Orchesella cincta* can be cultured in the laboratory has led to its nickname of the "*Drosophila*" of soil invertebrate zoologists (although *Folsomia candida* is another likely candidate when pollutants other than metals are considered). Verhoef *et al.* (1988) have shown that the preferred diet of *Orchesella cincta* is fungal mycelium, although it can be reared successfully on algae, the diet chosen by the Dutch group on which to feed their experimental animals.

*Orchesella cincta* assimilates 8.3% of the cadmium and 0.4% of the lead from a contaminated diet of algae, but is able to lose 30% of the assimilated cadmium and 48% of the assimilated lead at the following moult when the lining of the gut epithelium is shed (Joosse and Buker, 1979; Van Straalen and Van Meerendonk, 1987; Van Straalen *et al.*, 1987). This tissue contains metals in the form of intracellular granules (Humbert, 1978). Rates of assimilation

and excretion are both affected by temperature changes (Janssen and Bergema, 1991).

Physiological tolerance to lead and cadmium in *Orchesella cincta* has been shown by breeding experiments to have a genetic basis. Offspring of adults from clean areas grow less well on a metal-contaminated diet than offspring from adults from polluted areas (Posthuma, 1990). The physiological basis of the tolerance appears to be an increased excretion efficiency (Posthuma *et al.*, 1992).

Similar problems to the Swedish group have been experienced in trying to relate the findings of laboratory experiments to the situation in the field. Populations of *Orchesella cincta* exist in forest soils which are contaminated with cadmium to levels far above the "no effect level" for individual growth (Van Straalen *et al.*, 1989). Van Straalen and De Goede (1987) have suggested that the high natural mortality from predation obscures the sublethal effects on growth. It is clear that factors such as availability and choice of food, climate, and competition from other species may have a profound effect on the success or failure of an organism to resist pollution.

## 8.5 TRANSFER OF METALS TO PREDATORS OF COLLEMBOLA

For Collembola, as with many groups of soil (and other) animals, there is information on the identity of predators, but little quantitative data on the biomass consumed by particular species. Laboratory experiments using "model" food chains have been adopted to analyse specific pathways (e.g. yeast to the collembolan *Folsomia candida* to the carabid beetle *Nebria brevicollis*; Gruttke *et al.*, 1988) but relating this to the field is difficult.

Van Straalen (1987) has made the important point that because some species accumulate pollutants, more metal can be transferred from a prey population to predators than would be deduced from the transfer of biomass only. Thus in *Orchesella cincta*, because there is a tenfold increase in the concentration of lead during their lifetime, overall turnover of lead is about 1.5 times the turnover of biomass. In addition, because many predators are able to regulate their internal concentrations of metals (Hopkin, 1989), bioconcentration does not necessarily take place at higher trophic levels and predators are not subject to chronic poisoning due to long-term accumulation (Van Straalen and Ernst, 1991). However, this does not mean that predators are not affected by acute poisoning if they consume a single toxic dose from a highly contaminated individual prey item (Depledge, 1990; Hopkin, 1993a,b).

A wide range of arthropods prey on Collembola in soil and leaf litter. The major ones are centipedes, pseudoscorpions, spiders, carabid beetles, and harvestmen spiders. Birds may also consume Collembola. Thus, it is clear that Collembola provide a route of exposure to metals for their predators.

However, at present we are unable to set critical concentrations in Collembola that will protect their predators from poisoning because we lack the detailed ecological information on their biology in the field.

## 8.6 CONCLUSIONS

It may seem like an impossible task to set critical levels of metals in soils in the light of the complexities described in the previous sections of this review. However enormous progress has been made in understanding the dynamics of metals in soils in the past ten years. The new science of "ecotoxicology" has emerged which aims to describe the effects of environmental pollutants on natural populations (Calow, 1989). It is certain that many problems that seem insoluble today will be solved by the turn of the century.

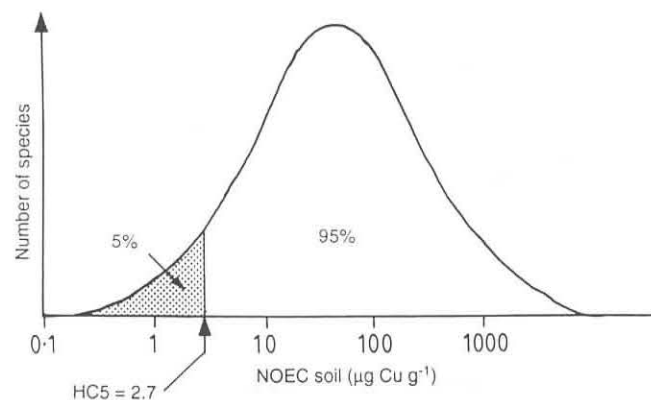
One of the most interesting developments has been the recognition that the toxicity of environmental pollutants may differ widely between species. Even within closely-related taxonomic groups such as terrestrial isopods, there are large differences in toxicity and rates of assimilation of metals between species (Van Straalen and Van Wensem, 1986; Hopkin, 1990b; Hames and Hopkin, 1991a; Van Straalen and Ernst, 1991). This may be due to selective feeding or differential rates of excretion resulting from metals being stored in different internal compartments that have different rates of turnover (Hames and Hopkin, 1991b; Figure 8.2). Furthermore some species apparently benefit from mild pollution (Bengtsson *et al.*, 1985b).

Thus, it is an impossible task to set a critical concentration for a pollutant in soil that will protect *all* species to the same degree. Indeed, the author has previously argued (Hopkin, 1990a) that the value for the critical concentration is largely a political decision as to what is an "acceptable level" of environmental damage. This theme was developed originally by Van Straalen and others who have promoted the idea of setting critical concentrations of metals in soils that will protect 95% of species (Van Straalen and Denneman, 1989). This 95% target has found favour with politicians, particularly in The Netherlands.

However, the present 95% protection values have been calculated on the assumption that the relationship between the number of species ( $y$  axis) and  $\log_{10}$  no observed effect concentrations (NOECs,  $x$  axis) is a normal curve (Figure 8.5). Since there have been very few experiments on NOECs, the left-hand region of the curve approaches zero. Consequently, the 95% protection levels suggested for cadmium, copper and lead by Van Straalen (1993) are extremely low (much lower than those of Bengtsson and Tranvik, 1989).

The "hazardous concentration for 5% of the species" (HC5) in soil for cadmium is only  $0.2 \mu\text{g Cd g}^{-1}$  and for lead is  $77 \mu\text{g Pb g}^{-1}$ . One would be hard-pressed to find soil anywhere in an industrialised country which contained levels of cadmium and lead that were below these values. Similarly,





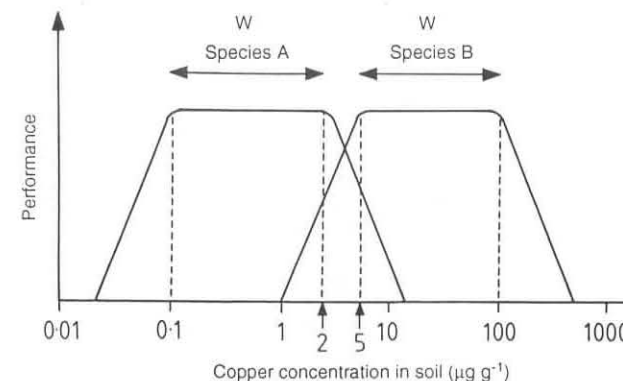
**Figure 8.5.** Schematic diagram of the proposed relationship between the number of species and the No Observed Effect Concentration (NOEC) of copper for soil invertebrates. Van Straalen (1993) has suggested that the hazardous concentration below which 5% of species are affected by copper poisoning (HC5) may be only  $2.7 \mu\text{g g}^{-1}$ .

the HC5 for copper is only  $2.7 \mu\text{g Cu g}^{-1}$  (Figure 8.5), about half the value that defines a copper-deficient soil for agricultural purposes ( $< 5 \mu\text{g Cu g}^{-1}$ ).

Van Straalen's figures are sure to be revised upwards as more experiments on NOECs are conducted (indeed the values contain an uncertainty margin due to the fact that only a small number of test species were used to estimate the distribution of sensitivities). However, if they are accurate, it is interesting to make two speculations as to the implications of these extremely low HC5 values. First, at least 5% of species are being affected by low level pollution of non-essential elements such as cadmium and lead in most soil habitats in areas which have hitherto been regarded as uncontaminated. Second, where essential elements such as copper are concerned, the NOEC level in soil for at least 5% of species may be below that which is needed to sustain the dietary requirements of the other 95% (Van Straalen, 1993).

The idea that some species in a habitat may be dying through copper poisoning while others are suffering from copper deficiency may seem rather far-fetched (Figure 8.6). However, many researchers measure total acid-soluble copper in leaf litter and soil without taking biological availability into account. Metals in soils exist in a wide range of abiotic compartments including pore water, organic acids (Kuiters and Mulder, 1992) and adsorbed onto humus and soil particles, the extent of which depends critically on pH (see Chapter 9, this volume). Biotic compartments include fungal hyphae and other living and dead microorganisms, Protozoa, plant roots, and invertebrates and their faecal material.

As has been shown in previous sections of this review, the soil and leaf litter "black box" contains fungal hyphae in which concentrations of copper may



**Figure 8.6.** Schematic diagram of the relationships between performance (fecundity, growth, survival) and concentration of copper in soil for two species of soil invertebrate. Species A falls within the HC5 category (Figure 8.5); its performance is reduced by concentrations of copper in soil of only  $2 \mu\text{g g}^{-1}$ . This level is insufficient to supply the minimum dietary requirement of species B ( $5 \mu\text{g g}^{-1}$ ). Note that the "windows of essentiality" (W) for the two species do not overlap.

be more than an order of magnitude higher than the substrate on which they are growing. It is not inconceivable that hyphae of some fungi contain very low concentrations of copper (possibly as "nutritional defence" as has been suggested for birch-feeding insects by Haukioja *et al.* (1991)). Fungivores that have evolved to eat these hyphae may not be able to tolerate mild enrichment of their diet with copper and may be killed by concentrations that would be insufficient to supply the dietary requirements of the majority of soil invertebrates (Hopkin, 1993c). In terms of other trace elements, competition between fungal species for iron is well established. One species may outcompete the other because it produces siderophores that bind all freely-available iron (Weinberg, 1984; Misaghi *et al.*, 1988).

Complications also arise when deciding which components of the ecosystem are most suitable in which to set critical concentrations. To date, most legislators have recommended critical levels of pollutants for abiotic components such as air, water, soil or sediment. However, setting a critical concentration for a 1 g sample of soil assumes that there is a close relationship between the level of the pollutant in soil and effects on the biota. This level is only directly relevant to an animal that consumes soil at a rate of about 1 g per day. For other species, the relationship may not be close at all (see, e.g. Jones and Hopkin, 1991; Hopkin, 1993b). Thus, to protect a particular species effectively, critical concentrations must be determined in the diet of that species. For fungivorous Collembola this may be the concentration in leaf litter above which the hyphae growing on its surface contain harmful levels of a pollutant.



Setting NOECs for soil animals is very difficult if other factors are to be taken into account. These include seasonal changes (Janssen and Bedaux, 1989; Janssen *et al.*, 1990, 1991; Janssen and Bergema, 1991) and the complexity of the interactions that occur between organisms in soil and leaf litter (Read *et al.*, 1987; Bengtsson *et al.*, 1988a; Verhoef and Brussard, 1990; Ponge, 1991a,b; Wilke, 1991). It is important to remember that most harmful effects may occur when the additional stress due to pollution combines with other stresses (e.g. cold or drought) that the animal would normally tolerate (i.e. the "straw that breaks the camel's back" theory of stress; see Welden and Slauson (1986), Sibly and Calow (1989) and Hopkin (1990a) for further definitions and discussions of stress). Furthermore the common toxicological practice of dividing the NOEC by 10 to obtain an acceptable safety margin for dietary exposure is not possible with essential elements such as copper and selenium because their "window of essentiality" is relatively small (Williams, 1981).

An alternative approach to setting critical concentrations for metals in soil would be to determine critical levels in a suite of biological indicator organisms (Samiullah, 1990). One could then establish relationships between concentrations in organisms that accumulate metals, and effects on others that do not (Hopkin, 1993b). It has been shown in an earlier publication that the concentrations of cadmium in the snail *Helix aspersa* can be predicted more accurately from the concentrations in the isopods *Oniscus asellus* or *Porcellio scaber* at metal-contaminated sites than from soil (Jones and Hopkin, 1991). In the future it may be possible, following cross-species laboratory experiments, to set critical concentrations for metals in *Porcellio scaber* that experiments have shown will protect 95% of other soil organisms (including Collembola) from poisoning (Hopkin *et al.*, 1993). Since isopods have a fairly cosmopolitan diet of fungal hyphae, leaf litter and other plant material (Hopkin, 1991), this approach would be more relevant than setting a critical concentration for soil that does not take into account biological availability. It would also go some way towards satisfying the desire to improve the links between laboratory and field studies (Anderson *et al.*, 1991), the "Holy Grail" of soil (and other) ecologists.

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